Institute of Marine Science

UNIVERSITY OF MIAMI

FINAL REPORT

GENERAL BIOLOGY OF PHYSALIA

September 1964

OFFICE OF NAVAL RESEARCH
DEPARTMENT OF THE NAVY

Contract Nonr 840 (17)

MIAMI, FLORIDA 33149

CLEARINGHOUSE FOR FEDERAL SCIENTIFIC AND TECHNICAL INFORMATION CFSTI
DOCUMENT MANAGEMENT BRANCH 410.11

LIMITATIONS IN REPRODUCTION QUALITY

ACCESSION # AD 606686

☒ 1. WE REGRET THAT LEGIBILITY OF THIS DOCUMENT IS IN PART UNSATISFACTORY. REPRODUCTION HAS BEEN MADE FROM BEST AVAILABLE COPY.

☐ 2. A PORTION OF THE ORIGINAL DOCUMENT CONTAINS FINE DETAIL WHICH MAY MAKE READING OF PHOTOCOPY DIFFICULT.

☐ 3. THE ORIGINAL DOCUMENT CONTAINS COLOR, BUT DISTRIBUTION COPIES ARE AVAILABLE IN BLACK-AND-WHITE REPRODUCTION ONLY.

☐ 4. THE INITIAL DISTRIBUTION COPIES CONTAIN COLOR WHICH WILL BE SHOWN IN BLACK-AND-WHITE WHEN IT IS NECESSARY TO REPRINT.

☐ 5. LIMITED SUPPLY ON HAND: WHEN EXHAUSTED, DOCUMENT WILL BE AVAILABLE IN MICROFICHE ONLY.

☐ 6. LIMITED SUPPLY ON HAND: WHEN EXHAUSTED DOCUMENT WILL NOT BE AVAILABLE.

☐ 7. DOCUMENT IS AVAILABLE IN MICROFICHE ONLY.

☐ 8. DOCUMENT AVAILABLE ON LOAN FROM CFSTI (TT DOCUMENTS ONLY).

☐ 9.

PROCESSOR: [signature]

TSL-107-10/64

THE MARINE LABORATORY
Institute of Marine Science
University of Miami

FINAL REPORT

Submitted to:

Office of Naval Research
Department of the Navy

GENERAL BIOLOGY OF PHYSALIA

By

Charles E. Lane

Contract Nonr 840 (17)

F. G. Walton Smith,
Director

enbacker Causeway

Florida 33149

64

BLANK PAGE

GENERAL BIOLOGY OF *PHYSALIA*

Final Report Covering the Period

From August, 1960 through July, 1964

The objective of this Program during the period covered by this report was to investigate the general biology of *Physalia*. It was proposed to concentrate research efforts on two major aspects of the general problem: first, the secretion of gas into the float, and second, the nature and circulation of the gastrovascular fluid. The results and our conclusions relating to the composition and mode of formation of the gas within the pneumatophore were described in a report to the Office of Naval Research in May 1962, and in the paper by Clark and Lane (1). In this report, attention was directed to the unexpected composition of the gas, consisting up to 15% of carbon monoxide. Various attempts were made to study the rate of secretion of the gas under various conditions.

The organism has been particularly resistant to all our attempts to maintain it successfully in culture, hence, any long time experimental attack on the problem of gas secretion has been prevented.

Gas in the float occurs under pressure of only a few millimeters of water above atmospheric pressure. On rare occasions, *Physalia* have been observed to valve out gas, permitting the organism to sink below the surface. Re-inflation of such forms has not been observed.

The composition of float gas varies considerably from organism to organism, suggesting that gas secretion is probably discontinuous in nature and suggesting further that secreted gas is rapidly diluted by diffusion of atmospheric components into the float. The composition of float gases in a series of animals is shown in Table I. These analyses were done with the Scholander one-half cubic centimeter gas analysis apparatus. Gas was withdrawn by hypodermic syringe from submerged individuals to avoid contamination with atmospheric air. The syringe barrels were well-oiled in advance of analysis. The gas was transferred under positive pressure into gas sampling bottles where it was maintained under mercury. Duplicate analyses of the same samples varied less than 5%.

Since suitable methods for maintaining Physalia under laboratory conditions could not be established, we were unable to investigate the nature of precursor materials involved in gas secretion in vivo, and all our attempts at an in vitro cultivation of gas gland were unsuccessful. It is apparent that Physalia and certain other Siphonophores illustrate an unusual biochemical sequence among animals, leading to the secretion of carbon monoxide against significant hydrostatic pressure. The mechanism of secretion and a detailed biochemical pathway for this synthesis remain to be described.

Gastrovascular fluid

The individual polyps of Physalia are interconnected by a common gastrovascular cavity (Fig. 1), lined throughout by

gastrodermal epithelium. This space is an extension of the cavity in the functional gastrozooids through which it communicates with surrounding sea water. During feeding, prey is ingested by the gastrozooids and is partly dissolved by extracellular hydrolytic enzymes. The gastrozooid contains a characteristic valvular structure at its base consisting of three to five interdigitating mesogleal plates, covered by gastrodermis (Fig. 1). The spaces between these plates in fixed material averages about 50μ. Particulate meterial larger than 50μ is probably retained in the gastrozooid. Smaller particles may be flushed through this "filter" into the general gastrovascular cavity where they are ingested by phagocytic gastrodermal cells. Terminal stages of digestion are thus completed intracellularly.

In the living animal the longer fishing tentacles display a continuous cycle of alternating contraction and relaxation. Each tentacle is hollow throughout its length. The cavity communicating with the general gastrovascular cavity is filled with gastrovascular fluid. As one tentacle contracts, gastrovascular fluid is forced from it into the central reservoir and thence into other tentacles. Extension of the tentacle is thus accomplished largely by hydraulic means. In this way, a primitive kind of circulation is established by which gastrovascular fluid is pumped from tentacle to tentacle and through the connecting gastrovascular spaces of the entire organism.

Since gastrovascular fluid pervades the entire animal, it became of some considerable interest to us to determine its composition. Gastrovascular fluid may be extracted from Physalia by inserting a micropipette into the lumen of a fishing tentacle. This is an operation of considerable delicacy, since the diameter of this space rarely exceeds 50μ at the base of the float. A major reservoir, however, separates the tissue components of the float and provides access to milliliter quantities of gastrovascular fluid. Collected gastrovascular fluid was quickly frozen on solid carbon dioxide and stored at -10° C in sealed containers until it could be analyzed. Na, K, Ca, and Mg were determined by flame photometry. Osmotic determination were done cryoscopically on samples of about 10 μl. Chromatographic analyses of gastrovascular fluid included ascending one-dimensional separations on paper and amino acid separations on columns of ion exchange resins. A micro-Kjeldahl method was employed for total nitrogen. Horizontal electrophoresis was conducted in acrylamide gum in 0.1 M phosphate buffer at pH 6.8 for 105 minutes at 350 V and 120 mA. The proximate composition of Physalia gastrovascular fluid is shown in Table II, free amino acid composition in Table III, and inorganic composition in Table IV.

Representative one-dimensional chromatograms of untreated whole gastrovascular fluid are shown in Fig. 2, and a representative amino acid spectrum is shown in Fig. 3.

When first withdrawn Physalia gastrovascular fluid has a faint bluish color. This color is discharged when the sample is shaken with nitrogen, and returns on aeration. This observation, combined with the high concentration of copper shown in Table IV, suggested a copper-protein complex, similar to hemocyanin. Samples of gastrovascular fluid were dialyzed against distilled water and the copper concentrations of the solutions on either side of the dialysis membrane were separately determined. Copper occurred in equal concentrations in the two solutions. Thus if a copper-protein complex does occur it is easily disrupted. Certainly no bonding such as is found in hemocyanin occurs in Physalia gastrovascular fluid. It is unlikely that a respiratory pigment such as hemocyanin plays any essential role in the normal physiology of Physalia. Table IV shows the iron content of gastrovascular fluid to be considerably higher than in sea water. When samples of gastrovascular fluid were examined by emission spectrography, exceedingly high concentrations of boron were observed.

Pharmacology of Physalia toxin

Our earlier studies (Lane and Dodge[2], Lane[3], Lane et al[4], and Lane[5]) were limited to a determination of acute toxicity after intravenous injection of toxin solution in mice or other experimental animals. The recent acquisition of a Grass polygraph has made it possible for the first time to dissect this acute toxicity by measuring various physiological parameters in experimental animals. Because of their larger size, we have

employed the laboratory rat as a test animal. Specimens between 200 and 250 grams weight are anesthetized with Nembutal and immobilized on a suitable rack. Electrocardiographic electrodes are attached to both forelimbs and to the left rear leg. The external jugular vein is exposed and toxin administered directly into this. Various dosages between 65 and 500 mg/kilo have been administered to a series of 27 rats. The general result of such injection is a functional disruption of the conducting system of the heart occurring within 3-5 seconds of completion of the injection, leading first to a functional separation of the atrium and ventricle, suppression of the P wave, various rapidly transient anomalies in the QRS complex, and terminal ventricular fibrillation (Fig. 4). This observation on the rats suggests a possible explanation for the single human fatality that we have so far been able to authenticate following a Physalia episode in man.

When Physalia toxin in solution is permitted to stand at room temperature, it loses 75% of its initial biological activity within 6 hours. This suggests that our crude toxin preparation may also contain an enzyme system which destroys it under proper conditions of temperature and dilution. Our present studies seek to test this hypothesis. When crude lyophilized toxin is treated with dinitrofluorobenzene and the resulting product is chromatographed on paper, a single amino terminal amino acid is detected. This terminal amino group in what must be a straight chain peptide is arginine.

Future studies will include the enumeration and identification of terminal amino groups in toxin after inactivation in solution at room temperature, to establish whether additional amino terminal groups are uncovered. The effects of various enzyme inhibitors will also be investigated.

LITERATURE CITED

1. Clark, Fred E., and Charles E. Lane. 1961. Composition of float gases of Physalia physalis. Proc. Soc. Exp. Biol. & Medicine, Vol. 107, 673-674.
2. Lane, Charles E., and Eleanor Dodge. 1958. The toxicity of Physalia nematocysts. Biol. Bull., Vol. 115, No. 2, 219-226.
3. Lane, Charles E. 1960. The toxin of Physalia nematocysts. Ann. N. Y. Acad. of Sci., Vol. 90, Article 3, 742-750.
4. Lane, Charles E., Bradner W. Coursen, and Kenneth Hines. 1961. Biologically active peptides in Physalia toxin. Proc. Soc. Exp. Biol. & Medicine, Vol. 107, 670-672.
5. Lane, Charles E. 1961. Physalia nematocysts and their toxin. In: The Biology of Hydra (H. M. Lenhoff and W. F. Loomis, eds.), pp. 169-178. University of Miami Press, Miami.

TABLE I

COMPOSITION OF FLOAT GAS OF PHYSALIA

SAMPLE	% O_2	% CO	%N
1	16.976	6.074	76.950
2	16.808	5.412	77.780
3	17.563	2.620	79.817
4	16.277	2.660	81.063
5	18.749	1.285	79.967
6	17.563	2.626	79.966
7	16.277	2.660	79.811
8	19.320	.712	79.968
9	20.775	.211	79.014
10	17.437	3.371	79.192
11	14.723	5.552	79.725
12	20.893	1.246	77.861
AVERAGE	17.78	2.89	79.26

TABLE II

AVERAGE PROXIMATE COMPOSITION

OF *PHYSALIA* GASTROVASCULAR FLUID

WATER		929.6 mg/ml
TOTAL SOLIDS:		
Ash	10.76 mg/ml	
Protein	28.75	
Free Amino Acids	8.75	
Unidentified	22.14	70.4

TABLE III

AVERAGE CONCENTRATION OF

AMINO ACIDS IN PHYSALIA

	TOXIN μmoles/mg	CAPSULE μmoles/mg	GASTROVASCULAR FLUID, μmoles/ml
CYS	Trace	Trace	0.199
ASP	.104	1.033	14.65
THR	.049	.876	8.82
SER	.072	.491	9.52
GLU	.715	1.069	15.22
PRO	.189	3.195	1.68
GLY	.161	7.445	11.99
ALA	.156	5.379	9.91
VAL	.070	1.065	4.43
CYST	.024	0.752	1.50
MET	.016	0.631	2.19
ILEU	.056	.719	4.30
LEU	.071	.559	5.65
TYR	.031	.199	1.80
PHE	.039	.228	3.28
NH_3	0.683	6.191	14.19
LYS	.040	.377	5.28
HIS	.120	.467	2.07
ARG	.027	.154	3.96
HYPRO	----	2.440	----

TABLE IV

COMPOSITION OF *PHYSALIA* GASTROVASCULAR FLUID

COMPARED WITH SEA WATER -- (mg/liter)

ELEMENT	SEA WATER	GASTROVASCULAR FLUID
Na	10,561	8,046
K	380	1,287
Ca	400	252
Mg	1,272	568
Sr	13.	39
Si	4.0	465
B	4.6	5.3
Al	0.5	34
Ba	0.05	<8.5
Fe	0.02	38
Zn	0.005	<8.5
Cu	0.01	5.3
Mn	0.01	0.71
Mo	0.002	~0.85
Ni	0.0005	<1.77
Ag	0.0003	<0.85
Co	0.0001	<0.85
V	0.0003	<0.85
Cr	Present	~1.77
Ti	Present	6.2

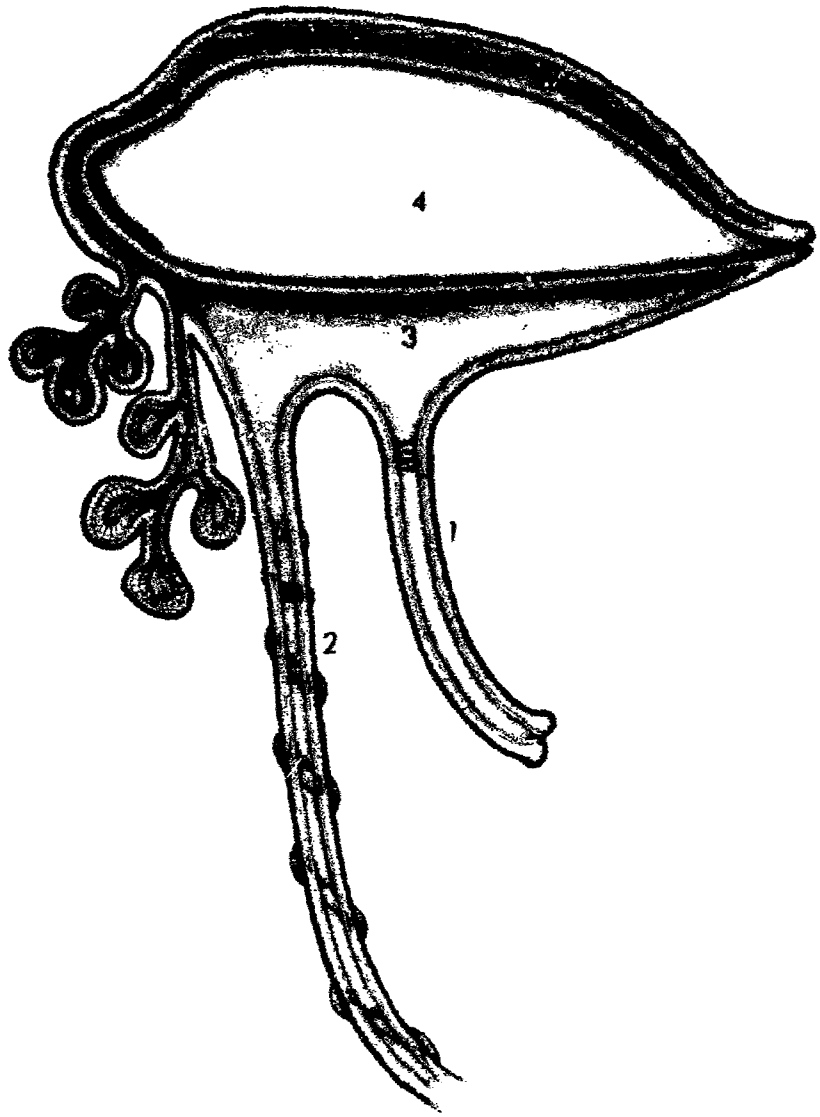

Fig. 1. Diagrammatic structure of Physalia showing:
(1) Gastrozooid; (2) Fishing tentacle;
(3) Gastrovascular cistern; (4) Pneumatophore.

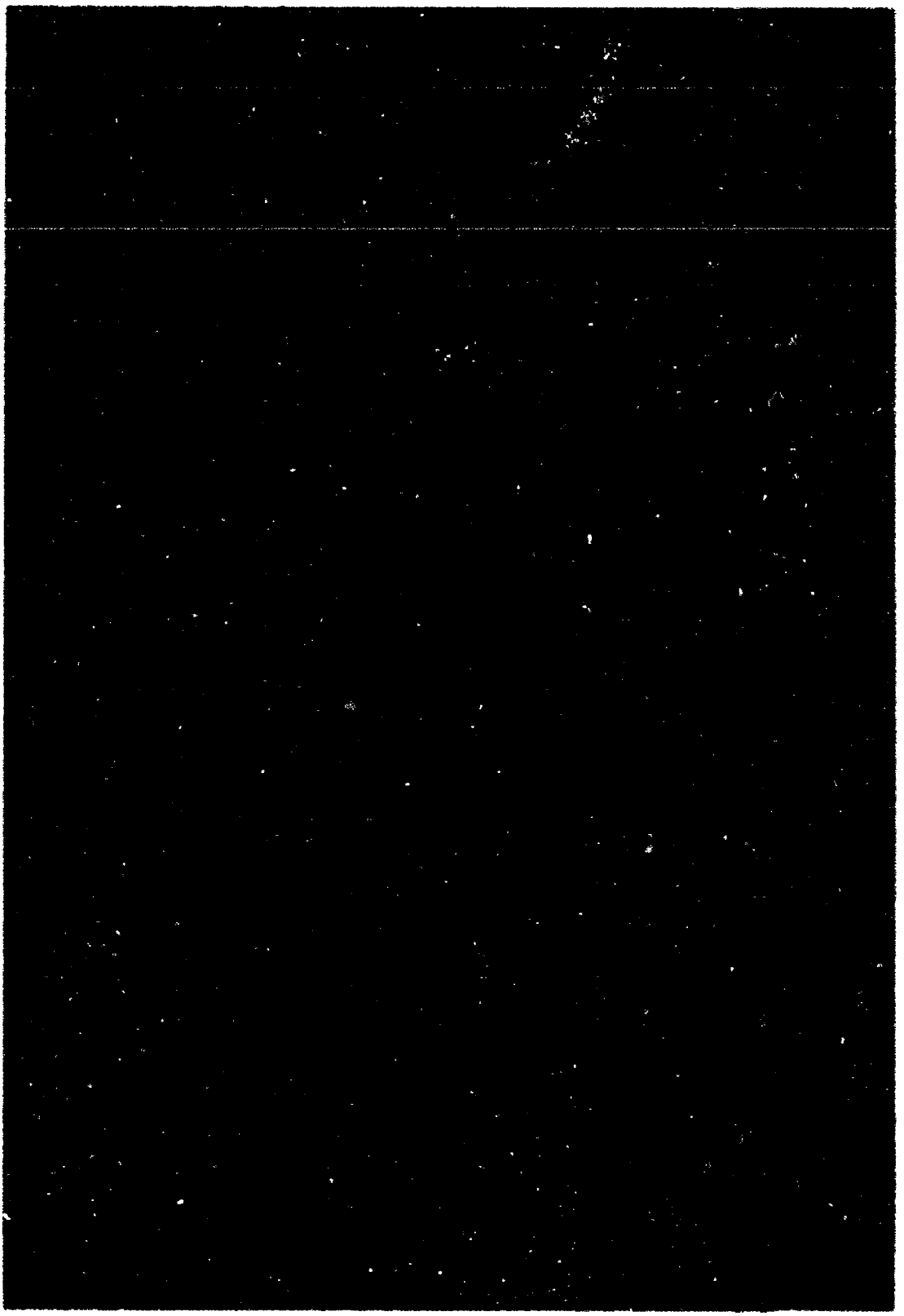

Fig. 2. One-dimensional chromatograms of separate untreated gastrovascular fluid samples.

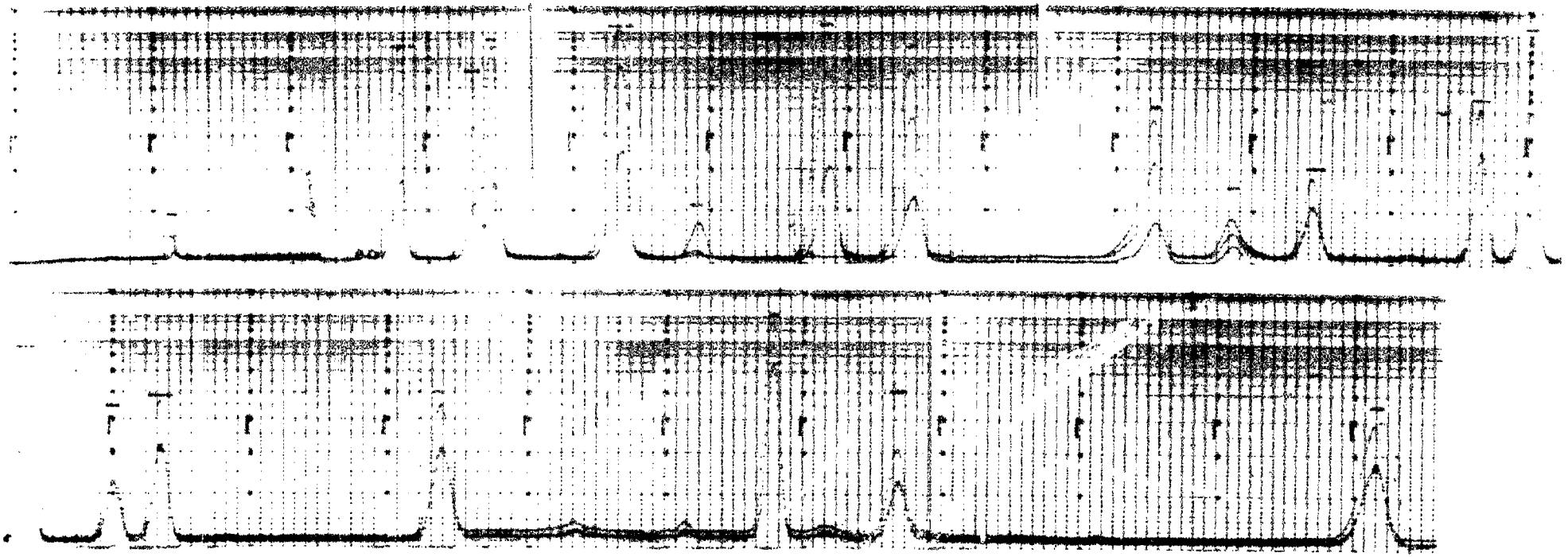

Fig. 3. Amino acids in Physalia gastrovascular fluid after hydrochloric acid hydrolysis.

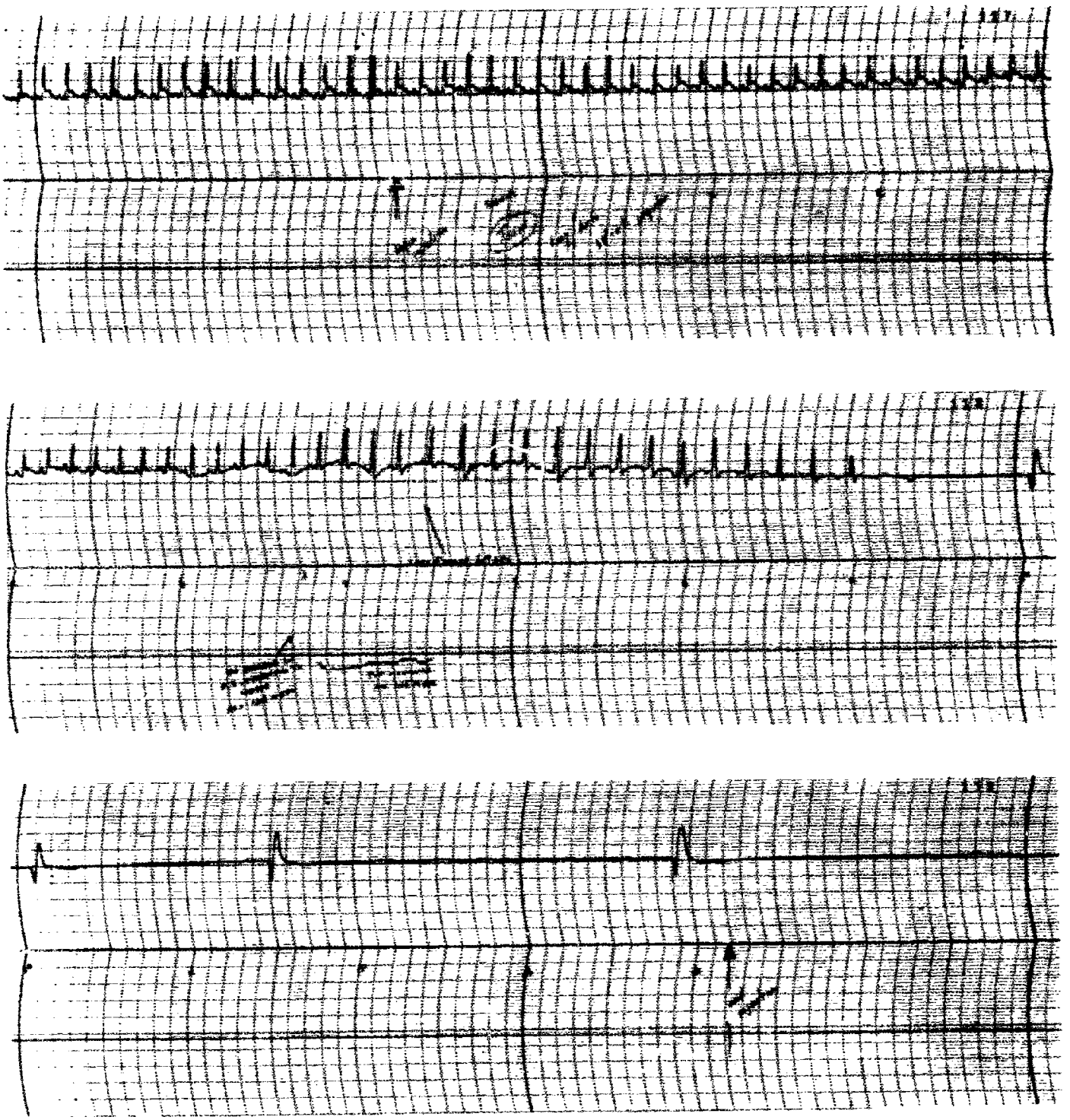

Fig. 4 The effects of intravenous injection of *Physalia* toxin into the rat. Dose level was 1 mg/kilo; the chart speed was 50 mm/sec.; the injection occurred between the arrows.

DISTRIBUTION LIST FOR
BIOLOGY BRANCH, OFFICE OF NAVAL RESEARCH
U. S. DEPT. OF THE NAVY

3 Office of Naval Research
Biology Branch (Code 446)
Washington 25, D. C.

6 Director, Naval Research Laboratory
Attention: Technical Information Officer
Washington 20, D. C.

1 Office of Technical Services
Department of Commerce
Washington 25, D. C.

1 Office of Naval Research Branch Office
10th Floor, John Crerar Library Bldg.
86 East Randolph Street
Chicago 1, Illinois

1 Office of Naval Research Branch Office
346 Broadway
New York 13, New York

1 Office of Naval Research Branch Office
1030 East Green Street
Pasadena 1, California

1 U. S. Navy Office of Naval Research
Branch Office Box 39, Navy No. 100
Fleet Post Office, New York, New York

1 ONR Contract Administrator, S. E. Area
2110 G. Street N. W.
Washington 7, D. C.

1 Director, Research Division
Bureau of Medicine and Surgery
Department of the Navy
Washington 25, D. C.

1 Commanding Office, Naval Research Inst.
National Naval Medical Center
Bethesda 14, Maryland

1 Commanding Officer, U.S. Naval Unit
Biological Division, Chemical Corps
Camp Detrick, Frederick, Maryland

1 Commanding Officer
U.S. Naval Ordnance Test Station
China Lake, California

1 Director, Office of Science
Office of Asst. Secretary of Def
Research and Engineering
Department of Defense
Washington 25, D.C.

1 Director, Div. of Medical Re
Chemical Warfare Laboratories
Army Chemical Center
Edgewood, Maryland

1 Commander, Air Force
Office of Scientific Research
Attention: Director, Biol. Scie
Washington 25, D. C.

1 The Director
National Library of Medicine
7th Street and Independence Ave.
Washington 25, D.C.

1 Director, Div. Grants & Fell
National Institutes of Health
Bethesda 14, Maryland

1 Dr. F. A. Chace
U. S. National Museum
Smithsonian Institution
Washington 25, D. C.

1 Chief, Branch of Fisheries I
U.S. Fish & Wildlife Service
Department of the Interior
Washington 25, D. C.

1 Director
Pacific Oceanic Fishery Investig
U.S. Fish & Wildlife Service
P.O. Box 3830
Honolulu,,Hawaii

1 Office of Technical Services
Department of Commerce
Washington 25, D.C.

1 Director, Att: Dr. B. Ketchu
Woods Hole Oceanographic Institut
Woods Hole, Massachusetts

e

arch

s Div.

W.

ships

BLANK PAGE

earch

ons

n

1 Director Bermuda Biological Station
St. George's West
Bermuda, British West Indies

1 Director: Att: Dr. D. Davenport
Institute of Marine Biology
University of Puerto Rico
Mayaguez, Puerto Rico

1 Chairman, Dept. of Zoology
Santa Barbara College
University of California
Goleta, California

8 American Institute of Biological Sciences
Advisory Committee on Biology
2000 P. Street N.W.
Washington 6, D. C.

1 Dr. Findlay E. Russell
College of Medical Evangelists
Los Angeles County General Hospital
Los Angeles 33, California

1 Dr. Paul R. Saunders
Dept. of Pharmacology & Toxicology
School of Medicine
University of Southern California
Los Angeles 7, California

1 Director: Att. Dr. A. Banner
Hawaii Marine Laboratory
University of Hawaii
Honolulu 14, Hawaii

1 Executive Secretary
Div. of Medical Sciences
National Research Council
2101 Constitution Ave. N.W.
Washington 25, D. C.

1 Director
Bingham Oceanographic Laborato
Yale University
Box 2025 Yale Station
New Haven, Connecticut

1 Dr. Ross F. Nigrelli
New York Zoological Society
New York Aquarium
185th St. & Southern Blvd.
New York 60, New York

20 Defense Documentation Center
Cameron Station
Alexandria, Virginia 22314